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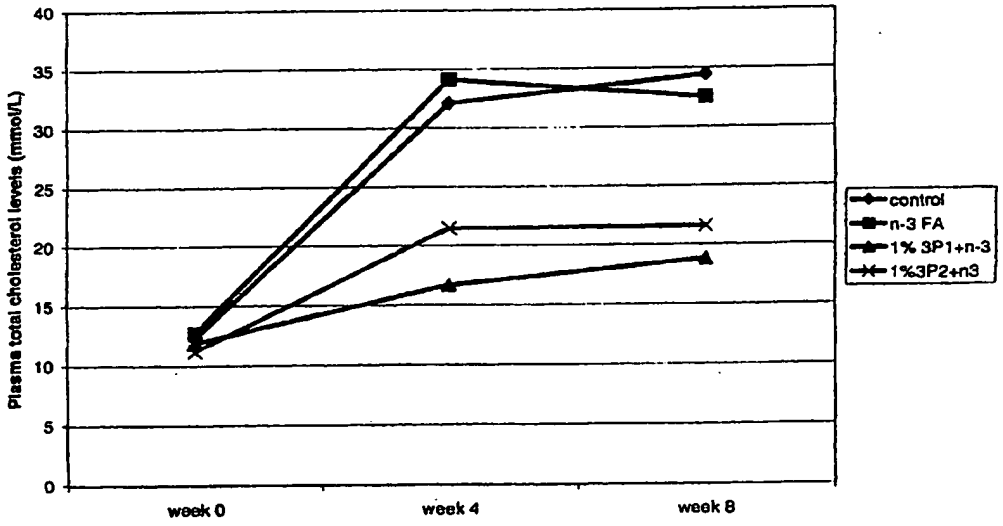

(57) Abstract

A composition for use in preventing and treating cardiovascular disease and other disorders comprises one or more phytosterols, phytostanols or mixtures of both, and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

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COMPOSITIONS COMPRISING PHYTOSTEROL, PHYTOSTANOL OR MIXTURES OF BOTH AND OMEGA-3 FATTY ACIDS OR DERIVATIVES THEREOF AND USE OF THE COMPOSITION IN TREATING OR PREVENTING CARDIOVASCULAR DISEASE AND OTHER DISORDERS

FIELD OF THE INVENTION

This present invention relates to the field of preventing and treating cardiovascular disease and other disorders using phytosterol-based compositions.

BACKGROUND OF THE INVENTION

While recent advances in science and technology are helping to improve quality and add years to human life, the prevention of atherosclerosis, the underlying cause of cardiovascular disease ("CVD") has not been sufficiently addressed. Research to date suggest that cholesterol may play a role in atherosclerosis by forming atherosclerotic plaques in blood vessels, ultimately cutting off blood supply to the heart muscle or alternatively to the brain or limbs, depending on the location of the plaque in the arterial tree (1,2). Overviews have indicated that a 1% reduction in a person's total serum cholesterol yields a 2% reduction in risk of a coronary artery event (3). Statistically, a 10% decrease in average serum cholesterol (e.g. from 6.0 mmol/L to 5.3 mmol/L) may result in the prevention of 100,000 deaths in the United States annually (4).

Sterols are naturally occurring triterpenoids that perform many critical cellular functions. Phytosterols such as campesterol, stigmasterol and beta-sitosterol in plants, ergosterol in fungi and cholesterol in animals are each primary components of cellular and sub-cellular membranes in their respective cell types. The dietary source of phytosterols in humans comes from plant materials i.e. vegetables and plant oils. The estimated daily phytosterol content in the conventional western-type diet is approximately 60-80 milligrams in contrast to a vegetarian diet which would provide about 500 milligrams per day.

Phytosterols hav received a great deal of attention du to their ability to decrease serum cholesterol levels when fed to a number of mammalian sp cies, including humans. While the precise mechanism of action remains largely unknown, the relationship between cholesterol and phytosterols is apparently due in part to the similarities between the respective chemical structures (the differences occurring in the side chains of the molecules). It is assumed that phytosterols displace cholesterol from the micellar phase and thereby reduce its absorption.

Over forty years ago, Eli Lilly marketed a sterol preparation from tall oil and later from soybean oil called Cytellin™ which was found to lower serum cholesterol by about 9% according to one report (5). Various subsequent researchers have explored the effects of sitosterol preparations on plasma lipid and lipoprotein concentrations (6) and the effects of sitosterol and campesterol from soybean and tall oil sources on serum cholesterols (7). A composition of phytosterols which has been found to be highly effective in lowering serum cholesterol is disclosed in PCT/CA95/00555 and comprises no more than 70% by weight beta-sitosterol, at least 10% by weight campesterol and stigmastanol. It is hypothesized in this patent application (which has already issued to patent in some countries) that there may be some form of synergy between the constituent phytosterols.

It is an object of the present invention to optimize the effects of phytosterols on CVD and other disorders.

SUMMARY OF THE INVENTION

The present invention provides a composition suitable for use alone or for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like which comprises one or more phytosterols or phytostanols or mixtures of both and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

The present invention further comprises foods, beverages, pharmaceuticals, nutraceuticals and the like which comprise a composition of one or more phytosterols, phytostanols or mixtures of both and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

The present invention furth r compris s a method of treating or preventing CVD and other disorders such as diabetes typ II, visceral obesity and hypertension in animals, including humans, by administering to the animal a composition which comprises one or more phytosterols, phytostanols or mixtures of both and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

The composition of the present invention has marked advantages over the phytosterol/stanol compositions previously known and described in the art. In particular and quite surprisingly, it has been found that there is an additive or synergistic effect between the phytosterol/stanol component and the omega-3 polyunsaturated fatty acid component of the composition on the absorption, catabolism and excretion of cholesterol and on the catabolism of triglycerides. These effects and other advantages are described in more detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing total cholesterol levels in plasma of 4 groups of the animals at baseline and during the experimental course (weeks 4 and 8); and

Figure 2 is a graph showing triglyceride levels in plasma of 4 groups of the animals at baseline and during the experimental course (weeks 4 and 8).

PREFERRED EMBODIMENTS OF THE INVENTION

According to one aspect of the present invention, there is provided a composition suitable for use alone or for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like which comprises one or more phytosterols, phytostanols or mixtures of both and one or more omega-3 polyunsaturated fatty acids or derivatives thereof. This composition has been found to have significant effects on the prevention and treatment of CVD and other disorders. In order to understand the possible mechanism of the synergy b tween the phytosterol/stanol component and the omega-3 polyunsaturated fatty acid

component of the composition, it is necessary to first outline what is known about phytosterols and their ffects on cholesterol and subsequently, what is known about omega-3 fatty acids vis-a-vis CVD.

While the precise mechanism of action is unclear, it is known that phytosterols have a beneficial effect on cholesterol homeostasis (transport, absorption, excretion and tissue distribution) in humans.

The intestine and the liver are the primary organs of cholesterol homeostasis in humans. The absorption of dietary cholesterol begins with lipids from the intestine. Cholesterol and fatty acids are then esterified in mucosal cells to form non-polar products and arranged with apoproteins to form chylomicrons. Chylomicrons enter the general circulation via the lymphatic system and are hydrolysed by plasma lipoprotein lipase into free fatty acids and monoglycerides. The dietary cholesterol transported in chylomicrons is delivered almost entirely to the liver as part of a chylomicron remnant which is then processed by hepatocyte cholesterol-7 alpha-hydroxylase into bile acids or excreted unmetabolized. Conversely, phytosterols are not endogenously synthesized in the animal body, therefore, are derived solely from the diet (originating from plants and edible oils) entering the body only via intestinal absorption. Within the intestine, cholesterol absorption is preferred over phytosterols absorption in mammals. For healthy humans, the absorption rate of phytosterols is usually less than 5% of dietary levels which is considerably lower than that of cholesterol which is over 40% (8 and 9). Thus approximately 95% of dietary phytosterols enter the colon. Only 0.3 to 1.7 mg/dl of phytosterols are found in human serum under normal conditions compared with daily dietary intakes of 160 to 360 mg\day but plasma levels have been shown to increase up to two-fold by dietary supplementation (10, 11 and 12). In summary, phytosterol serum levels are low due to poor phytosterol absorption and rapid elimination within the intestine.

As described herein, the term "extrinsic effect" refers to the role of phytosterols in the inhibition of cholesterol absorption by the enterocytes. One aspect of this mechanism is that phytosterols compete with cholesterol for the enterocyte shuttle transport from the gut lum n to the lymph or plasma. This transport requires intra-cellular re-assemblance of

cholesterol rich microparticle complexes with apoprotein B ("apo B"). In the enterocytes, phytosterols compete with cholesterol for apo B forming more lipophilic, apolar apo B complexes which cause shuttle inhibition and decrease lymphatic cholesterol content. If there is a decrease or absence of cellular synthesis of apo B, both serum phytosterol and cholesterols levels are low (i.e. diabetes type II, abetalipoproteinemia, and hypothyroidism). Changes in enterocyte shuttle selectivity, presumably due to an apo B mutation, could lead to high phytosterol and cholesterol levels (primary familial hypercholesterolemia and sitosterolemia).

As used herein, the term "systemic effect" refers to the effect of phytosterols on cholesterol bile acid synthesis, enterocyte and biliary cholesterol excretion, bile acid excretion, and changes in enzyme kinetics and cholesterol transport between various compartments within the body i.e. primary compartments such as the liver and enterocytes, secondary compartments such as organs, tissues and cells and tertiary compartments such as endothelial cells, monocytes and atherosclerotic plaque.

As in the enterocyte shuttle, phytosterols compete with cholesterol in the hepatic cells of the liver for elimination. In contrast with the enterocyte shuttle, however, the elimination of phytosterol via the bile route is faster than cholesterol. Correspondingly, the endogenous phytosterol pool size is low compared to cholesterol due to the combination of poor phytosterol intestinal absorption and faster biliary excretion.

The effects of omega-3 polyunsaturated fatty acids ("omega-3 PUFAs") on cholesterol levels were appreciated after comparison of saturated fats and unsaturated fats of vegetable origin on CVD. In feeding trials, the replacement of saturated fat and cholesterol in the diet by vegetable-based polyunsaturated fats caused changes that were associated with a reduced risk of CVD (13). These changes included marked reduction in low density lipoprotein ("LDL") cholesterol and very low density lipoprotein ("VLDL") cholesterol concentrations (14 and 15). The mechanism by which polyunsaturated fats reduce plasma cholesterol levels is still poorly understood, however, it has been suggested that it may be due to a decrease in cholesterol absorption in the gut lumen, a reduction in cholesterol synthesis in the body, a shift in cholesterol content from the plasma to other body compartm nts (th systemic effect), a change in the rate of

synthesis or catabolism of lipoproteins, depressed hepatic synthesis of fatty acids and triglycerides for LDL's, or an increase in the polyunsaturated fatty acid content of LDLs, which thereafter alters their structure so that they can only accommodate a smaller quantity of cholesterol (16, 17 and 18).

These findings led to recommendations that dietary saturated fat and cholesterol be replaced with polyunsaturated fatty acids ("PUFAs"), the principle families of which are the omega-3, omega-6 and omega-9 groups which are characterized by the position of the first double bond in the molecule i.e. omega-3 has the first double bond at the third carbon atom. These PUFAs, also referred to as "essential" fatty acids because they are not produced endogenously in animals, perform key functions in the body such as controlling plaque formation and blood pressure and promoting the body's defenses against cancer and infections. The deficiency of essential fatty acids in the body results in retarded body growth, scaly or broken skin, and excessive water loss from the body.

The omega-3 family of PUFAs (alpha-linolenic {18:3}, eicosapentaenoic acid {20:5} and docohexaenoic acid {22:6} occurs in very small amounts in most foods except fish and in other marine sources. Eicosapentaenoic acid ("EPA") and docohexaenoic acid ("DHA") actually originate in unicellular phytoplankton and seaweeds and, once incorporated into the lipids of fish and other marine animals that consume the algae, are passed on through the food chain to other species (19).

The benefits of omega-3 PUFAs is borne out by epidemiological studies showing a lower incidence of CVD in populations consuming a diet rich in fish or fish oils (20 and 21). More recently, researchers have postulated that there are two ways by which omega-3 PUFAs effect lipoprotein metabolism. The first involves acute or short term mechanisms such as the regulation of gene expression and the second involves long term effects including modification of cell membrane fluidity and lipoprotein physical characteristics.

Effect of Omega-3 PUFA on Lipoprotein Lipase and Apo C-III

Lipoprotein lipase ("LPL") is the major plasma triglyceride hydrolysing enzyme. LPL is synthesized in several cells and tissues including adipocytes, monocytes, liver, skeletal and cardiac muscle and hydrolyses lipoproteins into fr e fatty acids which are then

subsequently taken up by tissue where they are either oxidiz d to generate energy or re-esterified in triglycerides for storage (adipose tissue). Further, LPL has a role as a ligand for receptors, such as the LDL receptor-related protein ("LRP") and the VLDL receptor and as such contributes to the removal of lipoproteins from the circulation. Accordingly when LPL activity or synthesis is diminished for any reason, profound perturbations in plasma lipid concentrations may ensue.

Whereas LPL is a protagonist in the metabolism of triglycerides, apo C-II can be considered as an antagonist. Apo C-III is a 79 amino acid glycoprotein produced predominantly in the liver and the intestine. As a major component of plasma chylomicrons and VLDL, apo C-III delays the catabolism of these triglyceride-rich lipoproteins as well as the subsequent removal of the remnant particles from the plasma. In vitro, apo C-III has been shown to inhibit hydrolysis of triglycerides by LPL (22 and 23). It also inhibits the apo-E mediated clearance of lipoproteins by liver cells (24 and 25).

It has been found that fatty acids alter the transcription of the of the apo C-III and LPL genes via the peroxisome proliferator activated receptor ("PPAR") (26 and 27). Further studies show that treatment of hypertriglyceridemic patients with fibrates and omega-3 PUFAs (fish oil) results in the reduction of plasma triglyceride levels (28). Clinical reports have indicated that this hypotriglyceridemic effect is accompanied by both a decrease in the plasma concentration and synthesis rate of apo C-III (29) and an increase in LPL activity (30).

This facilitation of triglyceride catabolism by omega-3 PUFAs partly explains the desirable rise in high density lipoprotein ("HDL2") cholesterol, which is augmented by the partial inhibition of the lipid transfer protein (31).

Effect of Omega-3 PUFA on Hepatic Lipid Metabolism

Omega-3 PUFAs have been reported to alter hepatic lipid metabolism by several loci including:

1) increasing catabolism of chylomicron and chylomicron remnant triglycerides;

2) reducing hepatic triglyceride synthesis by the inhibition of enzymes acyl-coenzyme A: 1,2-diacylglycerol acyltransferase ("ADGAT") (32) or phosphatidate phosphohydrolase (33); or

3) inhibition of the incorporation of triglyceride (34) and/or inhibition of phosphatidylcholine synthesis (35), both of which are obligatory to the formation of VLDL. Reports also suggest that omega-3 PUFAs increase intra-cellular degradation of apo B which is necessary for the secretion of VLDL (36).

Effect of Omega-3 PUFA on LDL and VLDL Cholesterol

In one fatty fish feeding trial, LDL cholesterol concentrations decreased by 15% (37). In another trial, 24 grams of omega-3 PUFAs were consumed daily and a decrease of 20% in LDL cholesterol was noted. After kinetic studies of the metabolism of LDL, it was concluded that plasma LDL levels were lowered by a reduction in the rate of synthesis of apo B (38).

Also, VLDL cholesterol levels decreased with the consumption of fatty fish , dropping to about two-thirds of the initial values (39). This reduction has since been attributed to the reduced assembly and secretion of hepatic VLDL (40).

Synergy of Action

In the method of the present invention, CVD and many related disorders are treated and/or prevented by the adminstration to an individual of a composition comprising one or more phytosterols, phytostanols or mixtures of both and one or more omega-3 PUFAs. The effect of such combined administration on the factors underlying CVD, including incorrect cholesterol and triglyceride homeostasis, is greater than would have been expected. In other words, a synergistic effect, heretofore unappreciated, between the components of this composition has been found. The exact mechanism by which this synergy operates is unclear, although it is suspected that it is at least partially due to the different mechanisms by which phytosterols/stanols and omega-3 PUFAs effect the extrinsic cholesterol pathways i.e. the absorption, catabolism and excretion of

chylomicrons and chylomicron remnants and catabolism of triglycerides and also indirectly via cholesterol transfer protein transfer of HDL cholesterol.

In other words, it is very likely that this synergy between phytosterols/phytostanols and omega-3 PUFAs is based upon the relative enrichment by phytosterols/phytostanols of chylomicron and chylomicron remnants with t riglycerides (resulting in a decrease in the chylomicron cholesterol/triglyceride ratio) coupled with the increase by omega-3 PUFAs in the catabolism of triglycerides. This co-effect results in a decrease in postprandial lipaemia in humans by altering chlymicron composition, turnover and atherogenicity.

Phytosterols/Phytostanols

As used herein, the term "phytosterol" includes all phytosterols without limitation, for example: sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers. The term "phytostanol" includes all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers. It is to be understood that modifications to the phytosterols and phytostanols i.e. to include side chains also falls within the purview of this invention. It is also to be understood that this invention is not limited to any particular combination of phytosterols and/or phytostanols forming a composition. In other words, any phytosterol or phytostanol alone or in combination with other phytosterols and phytostanols in varying ratios as required depending on the nature of the ultimate formulation may incorporated with the omega-3 PUFAs. For example, the composition described in PCT/CA95/00555 which comprises no more than 70% by weight beta-sitosterol, at least 10% by weight campesterol and stigmastanol may be used within the scope of the present invention.

The phytosterols and phytostanols for use in this invention may be procured from a variety of natural sources. For example, they may be obtained from the processing of plant oils (including aquatic plants) such as corn oil and other vegetable oils, wheat germ oil, soy extract, rice extract, rice bran, rapeseed oil, sesame oil and fish oils. Without limiting the generality of the foregoing, it is to be understood that there are other sources of phytosterols and phytostanols such as marine animals from which the composition of the present invention may be prepared. US Patent Serial No. 4,420,427 teaches the

preparation of sterols from vegetable oil sludge using solvents such as methanol. Alternatively, phytosterols and phytostanols may be obtained from tall oil pitch or soap, by-products of forestry practises as described in PCT/CA95/00555, incorporated herein by reference.

Optionally, the phytosterol or phytostanol may be esterified prior to formation of the composition described herein. This esterification step renders the phytosterols and/or phytostanols more soluble in fats and oils which may, in some instances, facilitate the incorporation of the composition into various delivery systems.

To form phytosterol and/or phytostanol esters, one or more suitable aliphatic acids or their esters with low boiling alcohols are condensed with the phytosterol and/or phytostanol. A wide variety of aliphatic acids or their esters may be used successfully within the scope of the present invention and include all aliphatic acids consisting of one or more alkyl chains with one or more terminal carboxyl groups. These aliphatic acids may be natural of synthetic and are represented by the following chemical formulae:

a) R1-COOH (monocarboxylic acid) wherein:

R1 is an unbranched saturated alky group, represented by CH3-, CH3CH2- or CH3(CH2)nCH2- WHERE n=3-25; or
R1 is a branched saturated alkyl group represented by CnH2n+1-where n=1-25 is the number of carbon atoms contained in the group R1; the branching typically refers, but is not limited to one or more methyl group side chains (branches); or
R1 is an unbranched or branched unsaturated alkyl group, represented by the formula CnH2n-2m+1, where n=1-25 is the number of carbon atoms in R1 and m=degree of unsaturation; or

b) HOOC-R2-COOH is a dicarboxylic acid wherein:

R2 is an unbranched saturated alkly group, represented by - CH2-, or - CH2CH2-, or -CH2(CH2)nCH2 wher n=3-25; or
R2 is a branched saturated alkyl group represented by -CnH2n- where n=1-25 is

the number of carbon atoms contained in the group R2: the branching typically refers, but is not limited to, one or more methyl group side chains (branches); or R2 is an unbranched or branched unsaturated alkyl group, represented by the formula CnH2n-2m, where n=1-25 is the number of carbon atoms in R2 and m=degree of unsaturation; or

c) a tricarboxylic acid represented by the formula:

HOOC——R3——COOH

COOH

wherein, in this formula:

R3 is a branched saturated alkyl group represented by -CnH2n-1- where n=1-25 is the number of carbon atoms contained in the group R3; the branching typically refers, but is not limited to, one or more methyl group side chains (branches); or R3 is a branched unsaturated alkyl group, represented by CnH2n-2m-1- wherein n=1-25 is the number of carbon atoms in R3 and m= the degree of unsaturation; or

d) a mono-. di-, or tricarboxylic acid as defined above, which may contain one, two or three hydroxyl groups in the molecule.

In a preferred form, the aliphatic acid is either a straight-chain or branched unsaturated or saturated fatty acid selected, inter alia, from the following list:
valeric acid, isovaleric acid, sorbic acid, isocaproic acid, lauric acid, myrestic acid, palmitic acid, stearic acid, caproic acid, ascorbic acid, arachidic acid, behenic acid, hexacosanoic acid, octacosanoic acid, pentadecanoic acid, erucic acid, linoleic acid, linolenic acid, arachidonic acid, acetic acid, citric acid, tartaric acid, palmitoleic acid and oleic acid. The most preferable fatty acids within the scope of the present invention are linoleic acid, linolenic acid and arachidonic acid which may be obtained from natural sources such as safflower oil, sunflower oil, olive oil and corn oil (linoleic acid), safflower oil, sunflower oil, olive oil and jojoba oil (linolenic acid and arachidonic acid) and rapeseed oil (erucic acid).

A particular advantage in using fatty acids to form esterifed phytost rols or phytostanols , i.e. saturated fats, in accordance with the present invention lies in the fact that saturated fats increase lipoprotein lipase activity. The activity of this latter enzyme reduces visceral fat formation.

To form a phytosterol ester in accordance with the present invention, the selected phytosterol and aliphatic acid or its ester with volatile alcohol are mixed together under reaction conditions to permit condensation of the phytosterol with the aliphatic acid to produce an ester. A most preferred method of preparing these esters which is widely used in the edible fat and oil industry is described in US Patent Serial No. 5,502,045 (which is incorporated herein by reference). As no substances other than the free phytosterol, a fatty acid ester or mixture thereof and an interesterification catalyst like sodium ethylate are used, the technique is highly suitable for preparing products ultimately for human consumption. In overview, this preferred method, adapted for use within the present invention, comprises heating the phytosterol(s) with a vegetable oil fatty acid ester (preferably a methyl ester) at a temperature from 90-120°C and subsequently adding a suitable catalyst such as sodium ethylate. The catalyst is then removed/destroyed by any one of the techniques known in the art e.g. adding water and/or filtration/centrifugation.

Another method which may be used in accordance with the present invention is described in US Patent Serial No. 4,588,717, which is also incorporated herein by reference. A preferred method is to mix the phytosterol and the fatty acid together bringing the mixture to a temperature of from about 15°C to about 45°C at about atmospheric pressure for approximately one to three hours.

Omega-3 PUFAs

The omega-3 PUFAs for use within the composition of the present invention are selected from alpha-linolenic acid, EPA and DHA in the form of, inter alia, fatty acids, triglycerides, phospholipids, esters or free fatty acid salts. In one embodiment of the present invention, the omega-3 PUFAs may be extracted from zooplankton, fish or other marine animals using suitable bioconcentration techniques. In the alternative, omega-3 PUFAs may be

synthesized using microalgae as the source material. In on preferred form, marine fish oil may be mix d directly with th phytosterol and/or stanol components to form the composition of the present invention. The marine oil may be extracted by techniques known in the art from, inter alia: finfish such as cod, salmon, tuna, herring, halibut, shark, catfish, pollock, dogfish, anchovy, mackerel, trout, and eel; animals such as seals and whales; crustaceans such as crabs, clams and lobster; mollusks and the like.

Without limiting the generality of the foregoing, the most preferred marine sources of omega-3 PUFAs are as follows:

Source	Grams, Omega-3/100 calories*
fish oil capsules	2.86
salmon (sockeye)	1.71
tuna	1.22
salmon (pink)	1.15
shark (spiny dogfish	1.14
halibut	1.13
anchovy	1.10
salmon (Atlantic)	1.08
mackerel (Atlantic)	1.08
salmon (Pacific)	1.03
spanish sardine	0.91
trout (rainbow)	0.86
mackerel (Pacific)	0.85
swordfish (herring)	0.75

* (41)

Alternatively, plant sources of omega-3 PUFAs may be used. The great advantage of plant sources is reduced odour as compared to some marine sources. Plant sources include, but are not limited to, plant oils such as hemp oil, flax seed oil and corn oil as well as soy.

Although oth r ratios and concentrations are fully within the purview of the pr sent invention, (i.e. the inv ntion is not to be limited to the concentrations disclosed) it is preferred that the composition of this invention comprise, in a form for daily administration to humans, up to 6 grams of phytosterols and/or phytostanols and up to 3 grams of omega-3 PUFAs. In another preferred form, the composition comprises form 0.5 to 2.5 grams each of phytosterols and/or phytostanols and omega-3 PUFAs. In a most preferred form, the composition comprises 1.5 grams of each component:

Omega-6 PUFAs

Optionally, one or more omega-6 PUFAs may be added to the composition of the present invention either:

1) as one or more of the extraction products linoleic acid, gamma-linolenic acid or arachidonic acid or derivatives thereof; or
2) as one or more plant oils. Examples of suitable plant oils, include but are not limited to: vegetable oils, safflower oil, sunflower oil, hempseed oil, primrose oil, cottonseed oil, peanut oil and the like.

Safflower oil is a most preferred source of omega-6 PUFA as it has the highest linoleic acid content of any commercial oil. Other sources of omega-6 PUFAs are described in the following table, in which the omega-6 content is shown as a percentage of total fat.

Oil	Linoleic acid (Omega-6)
Butter fat	2
Coconut	2
Olive	8
Lard	11
Flax	14
Canola	21
Peanut	34
Soybean	54
Hemp	58
Corn	60
Sunflower	69
Safflower	78

Other Compon nts

Optionally, the composition of the present invention comprising phytosterol and/or phytostanol with omega-3 PUFAs may be combined with other components to enhance further the therapeutic and dietary efficacy. For example, the composition may comprise one or more of the following:

saturated fatty acids; other PUFAs; short, medium, long or very long chain fatty acids (saturated or unsaturated); neutral fats; cholesterol; esters and triacylglycerols.

Delivery Systems

Although it is fully contemplated within the scope of the present invention that the compositions may be administered to animals, particularly humans, directly and without any further modification, it is possible to take further steps to enhance delivery and ensure even distribution throughout the food, beverage, pharmaceutical, nutraceutical and the like to which they are added. Such enhancement may be achieved by a number of suitable means such as, for example, solubilizing or dispersing the elements of the composition to form emulsions, solutions and dispersions or self-emulsifying systems; lyophilizing, spray drying, controlled precipitating, or a combination thereof; forming solid dispersions, suspensions, hydrated lipid systems; forming inclusion complexations with cyclodextrins; and using hydrotopes and formulations with bile acids and their derivatives.

Each of the techniques which may be used in accordance with the present invention are described below.

Emulsions

Emulsions are finely divided or colloidal dispersions comprising two immiscible phases, e.g. oil and water, one of which (the internal or discontinuous phase) is dispersed as droplets within the other (external or discontinuous phase). Thus an oil-in-water emulsion consists of oil as the internal phase, and water as the discontinuous or external phase, the water-in-oil emulsion being the opposite. A wide variety of emulsified systems may be formed which comprise the compositions including standard emulsions, microemulsions and those systems which are self-emulsifying (emulsify on exposure to agitated aqueous fluids such as gastric or int stinal fluids).

Generally, emulsions may include oil and water phases, emulsifiers, emulsion stabilizers and optionally preservatives, flavouring agents, pH adjusters and buffers, chelating agents, antifoam agents, tonicity adjusters and anti-oxidants. Suitable emulsifiers (wherein bracketed numerals refer to the preferred HLB values) include: anionic surfactants such as alcohol ether sulfates, alkyl sulfates (30-40), soaps (12-20) and sulfosuccinates; cationic surfactants such as quaternary ammonium compounds; zwitterionic surfactants such as alkyl betaine derivatives; amphoteric surfactants such as fatty amine sulfates, difatty alkyl triethanolamine derivatives (16-17); and nonionic surfactants such as the polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated fatty acids and alkyphenols, water-soluble polyethyleneoxy adducts onto polypropylene glycol and alkyl polypropylene glycol, nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxyethanol, polyethylene glycol, octylphenoxy-polyethoxyethanol, lanolin alcohols, polyoxyethylated (POE) alkyl phenols(12-13), POE fatty amides, POE fatty alcohol ethers, POE fatty amines, POE fatty esters, poloxamers (7-19), POE glycol monoethers (13-16), polysorbates (17-19) and sorbitan esters (2-9). This list is not intended to be exhaustive as other emulsifiers are equally suitable.

Appropriate emulsion stabilizers include, but are not limited to, lyophilic colloids such as polysaccharides, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, tragacanth, xanthan gum; amphoterics (e.g. gelatin) and synthetic or semi-synthetic polymers (e.g. carbomer resins, cellulose ethers and esters, carboxymethyl chitin, polyethylene glycol-n (ethylene oxide polymer H(OCH2CH2)nOH); finely divided solids including clays (e.g. attapulgite, bentonite, hectorite, kaolin, magnesium aluminum silicate and montmorillonite), microcrystalline cellulose oxides and hydroxides (e.g. aluminum hydroxide. magnesium hydroxide and silica); and cybotactic promoters/gellants (including amino acids, peptides, proteins lecithin and other phospholipids and poloxamers).

Suitable anti-oxidants for use in the formation of emulsions include: chelating agents such as citric acid, EDTA, phenylalanine, phosphoric acid. tartaric acid and tryptophane; preferentially oxidized compounds such as ascorbic acid, sodium bisulfite and sodium sulfite; water soluble chain terminators such as thiols and lipid soluble chain terminators such as alkyl gallates, ascorbyl palmitate, t-butyl hydroquinone, butylated hydroxyanisole,

butylated hydroxytoluene, hydroquinone, nordihydroguaiaretic acid and alpha-tocopherol. Suitable preservatives, pH adjustment agents, and buffers, chelating agents, osmotic agents, colours and flavouring agents are discussed hereinbelow under "Supensions", but are equally applicable with respect to the formation of emulsions.

The general preparation of emulsions is as follows: the two phases (oil and water) are separately heated to an appropriate temperature, the same in both cases, generally 5-10°C above the melting point of the highest melting ingredients in the case of a solid or semi-solid oil, or where the oil phase is liquid, a suitable temperature as determined by routine experimentation). Water-soluble components are dissolved in the aqueous (water) phase and oil-soluble components, are dissolved in the oil phase. To create an oil-in water emulsion, the oil phase is vigorously mixed into the aqueous phase to create a suitable dispersion and the product is allowed to cool at a controlled rate with stirring. A water-in-oil emulsion is formed in the opposite fashion i.e. the water phase is added to the oil phase. When hydrophilic colloids are a part of the system as emulsion stabilizers, a phase inversion technique may be employed whereby the colloid is mixed into the oil phase rather than the aqueous phase, prior to addition to the aqueous phase. In using any phytosterol or phytostanol composition, it is preferred to add these to the oil phase prior to heating.

Microemulsions, characterized by a particle size at least an order of magnitude smaller (10-100 nm) than standard emulsions and defined as "a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid" (42), may also be formed comprising phytosterol or phytostanol compositions. In a preferred form, the microemulsion comprises a surfactant or surfactant mixture, a co-surfactant (usually a short chain alcohol) the chosen phytosterol or phytostanol and omega-3 PUFAs, water and optionally other additives.

This system has several advantages as a delivery system for the compositions of the present invention. Firstly, microemulsions tend to be created spontaneously, that is, without the degree of vigorous mixing required to form standard emulsions. From a commercial perspective, this simplifies the manufacturing process. Secondly, microemulsions may be sterilized using microfiltration techniques without breaking the

microstructure du to th small diameter of the microdroplets. Thirdly, microemulsions are highly thermodynamically stable. Fourthly, microemulsions poss ss high solubilizing power which is particularly important as they can further enhance the solubilization of the phytosterols/phytostanols.

Surfactant or surfactant mixtures which are suitable for use in the formation of microemulsions can be anionic, cationic, amphoteric or non-ionic and possess HLB (hydrophile-lipophile balance) values within the range of 1-20, more preferably in the ranges 2-6 and 8-17. Especially preferred agents are non-ionic surfactants, selected from the group consisting of polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated fatty acids and alkyphenols, water-soluble polyethyleneoxy adducts onto polypropylene glycol and alkyl polypropylene glycol, nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxyethanol, polyethylene glycol, octylphenoxy-polyethoxyethanol, lanolin alcohols, polyoxyethylated (POE) alkyl phenols (12-13), POE fatty amides, POE fatty alcohol ethers, POE fatty amines, POE fatty esters, poloxamers (7-19), POE glycol monoethers (13-16), polysorbates (10-17) and sorbitan esters (2-9).

There are a number of methods known and used by those skilled in the art for making microemulsions. In a preferred method of forming microemulsions of the present invention, a surfactant, a co-surfactant, and phytosterol and/or phytostanol plus the omega-3 PUFAs (pre-dissolved in a suitable proportion of an appropriate oil) are mixed and then titrated with water until a system of desired transparency is obtained.

In a further preferred embodiment, the formation of microemulsions may be achieved by mixing the phytosterol and/or phytostanol plus omega-3 components with hydrotropic agents and food-grade surfactants (refer to 43).

Solutions and Dispersions

Phytosterol and/or phytostanol omega-3 PUFA compositions may be dissolved or dispersed in a suitable oil vehicle, with or without additional excipients, and used in this form, for example, in general food usage, in basting meats and fish, and for incorporation into animal feeds.

Suitable solubilizing agents include all food grade oils such as plant oils, marine oils (such as fish oil) and vegetable oils, monoglycerides, diglycerides, triglycerides, tocopherols and the like and mixtures thereof.

Self-Emulsifying Systems

The compositions of the present invention may be mixed with appropriate excipients, for example, surfactants, emulsion stabilizers (described above) and the like, heated (if necessary) and cooled to form a semi-solid product capable of forming a spontaneous emulsion on contact with aqueous media. This semi-solid product may be used in numerous other forms such as filler material in two-piece hard or soft gelatin capsules, or may be adapted for use in other delivery systems.

Solid Dispersions

An alternative means of further increasing the solubility/dispersability of the compositions of the present invention involves the use of solid dispersion systems. These dispersions may include molecular solutions (eutectics), physical dispersions or a combination of both.

For example, solid dispersions may typically be prepared by utilizing water-soluble polymers as carriers. Without limitation, these carriers may include, either alone or in combination: solid grade polyethylene glycols (PEG's), with or without the addition of liquid grade PEG's; polyvinylpyrrolidones or their co-polymers with vinyl acetate and cellulose ethers and esters. Other excipients, such as additional members of the glycol family e.g. propylene glycol, polyols, e.g. glycerol etc.. may also be included in the dispersions.

Solid dispersions may be prepared by a number of ways which are familiar to those in the art. These include, without limitation, the following methods:

(a) fusing the ingredients, followed by controlled cooling to allow solidification and subsequent mechanical grinding to produce a suitable powder. Alternatively, the molten (fused) dispersion may be sprayed into a stream of cooled air in a spray drier to form solid particles (prilling) or passed through an extruder and

spheroniser to form solid masses of a controlled particle size. In a further alt rnative, the molten dispersion is filled directly into two-piece hard gelatin capsules;

(b) dissolving the ingredients in a suitable solvent system (organic, mixed organic, organic-aqueous) and then removing the solvents e.g. by evaporating at atmospheric pressure or in vacuo, spray drying, lyophilizing and the like; or, in a variation of the foregoing, and

(c) dissolving the ingredients in a suitable solvent system, subsequently precipitating them from solution by the use of an immiscible solvent in which the ingredients have little or no solubility, filtration, removing the solvent, drying and optionally grinding to provide a suitable powder form.

Suspensions

Suspensions, which may be used to enhance further the solubility and/or dispersability of the compositions , comprise a solid, perhaps finely divided, internal phase dispersed in an oily or aqueous external phase (the vehicle). In addition, the solid internal phase may be added to an emulsion as described above during its' formation to produce a delivery system having properties common to both suspensions and emulsions.

Numerous excipients, which are commonly used in the art, may be suitable for producing a suspension within the scope of the present invention. Typically, a suspension comprises an oily or aqueous vehicle, the dispersed (suspended) internal phase, dispersing and/or wetting agents (surfactants), pH adjustment agents/buffers, chelating agents, antioxidants, agents to adjust ionic strength (osmotic agents) colours, flavours, substances to stabilize the suspension and increase viscosity (suspending agents) and preservatives.

Appropriate vehicles include, but are not limited to: water, oils, alcohols, polyols, other edible or food grade compounds in which the phytosterol composition is partially or not soluble and mixtures thereof. Appropriate dispersing agents include, but are not limited to: lecithin; phospholipids; nonionic surfactants such as polysorbate 65, octoxynol-9,

nonoxynol-10, polysorbate 60, polysorbat 80, polysorbate 40, poloxamer 235, polysorbate 20 and poloxamer 188; anionic surfactants such as sodium lauryl sulfate and docusate sodium; fatty acids, salts of fatty acids, other fatty acid esters, and mixtures thereof.

Agents/buffers for pH adjustment include citric acid and its salts, tartaric acid and its salts, phosphoric acid and its salts, acetic acid and its salts, hydrochloric acid, sodium hydroxide and sodium bicarbonate. Suitable chelating agents include edetates (disodium, calcium disodium and the like), citric acid and tartaric acid. Suitable antioxidants include ascorbic acid and its salts, ascorbyl palmitate, tocopherols (especially alpha-tocopherol), butylated hydroxytoluene, butylated hydroxyanisole, sodium bisulfite and metabisulfite. Suitable osmotic agents include monovalent, divalent and trivalent electrolytes, monosaccharides and disaccharides. Suitable preservatives include parabens (Me, Et, Pr, Bu and mixtures thereof), sorbic acid, thimerosal, quaternary ammonium salts, benzyl alcohol, benzoic acid, chlorhexidine gluconate and phenylethanol. Colours and flavours may be added as desired and may be selected from all natural, nature-identical and synthetic varieties.

Hydrated Lipid Systems

In a further embodiment of the present invention, the solubility/dispersability of the compositions of the present invention may be further enhanced by the formation of phospholipid systems such as liposomes and other hydrated lipid phases. by physical inclusion. This inclusion refers to the entrapment of molecules without forming a covalent bond and is widely used to improve the solubility and subsequent dissolution of active ingredients.

Hydrated lipid systems, including liposomes, can be prepared using a variety of lipid and lipid mixtures, including phospholipids such as phosphatidylcholine (lecithin), phosphodiglyceride and sphingolipids, glycolipids, and the like. The lipids may preferably be used in combination with a charge bearing substances such as charge-bearing phospholipids, fatty acids, and potassium and sodium salts thereof in order to stabilize the resultant lipid systems. A typical process of forming liposomes is as follows:

1) dispersion of lipid or lipids and the phytosterols and/or phytostanols along with the

omega-3 PUFAs in an organic solvent (such as chloroform, dichloromethane, ether, ethanol or other alcohol, or a combination thereof). A charged species may be added to reduce subsequent aggregation during liposome formation. Antioxidants (such as ascorbyl palmitate, alpha-tocopherol, butylated hydroxytoluene and butylated hydroxyanisole) may also be added to protect any unsaturated lipids, if present;

2) filtration of the mixture to remove minor insoluble components;

3) removal of solvents under conditions (pressure, temperature) to ensure no phase separation of the components occur;

4) hydration of the "dry" lipid mixture by exposure to an aqueous medium containing dissolved solutes, including buffer salts, chelating agents, cryoprotectorants and the like; and

5) reduction of liposome particle size and modification of the state of lamellarity by means of suitable techniques such as homogenization, extrusion etc..

Any procedure for generating and loading hydrated lipid with active ingredients, known to those skilled in the art, may be employed within the scope of this invention. For example, suitable processes for the preparation of liposomes are described in references 44 and 45, both of which are incorporated herein by reference. Variations on these processes are described in US Patent Serial No. 5,096,629 which is also incorporated herein by reference.

US Patent Serial No. 4,508,703 (also incorporated herein by reference) describes a method of preparing liposomes by dissolving the amphiphillic lipidic constituent and the hydrophobic constituent to form a solution and thereafter atomizing the solution in a flow of gas to produce a pulverent mixture.

Cyclodextrin Complexes

Cyclod xtrins are a class of cyclic oligosaccharide molecules comprising glucopyranose

sub-units and having a toroidal cylindrical spatial configuration. Commonly availabl members of this group comprise molecules containing six (alpha-cyclodextrin), seven (beta-cyclodextrin) and eight (gamma-cylcodextrin) glucopyranose molcules, with the polar (hydrophilic) hydroxyl groups oriented to the outside of the structure and the apolar (lipophilic) skeletal carbons and ethereal oxygens lining the interior cavity of the toroid. This cavity is capable of accomodating (hosting) the lipophilic moiety of an active ingredient (the guest molecule, here the composition of the present invention) by bonding in a non-covalent manner to form an inclusion complex.

The external hydroxyl substituents of the cyclodextrin molecule may be modified to form derivatives having improved solubility in aqueous media along with other desired enhancements, such as lowered toxicity, etc.. Examples of such derivatives are: alkylated derivatives such as 2,6-dimethyl-beta-cyclodextrin; hydroxyalkylated derivatives such as hydroxypropyl-beta-cyclodextrin; branched derivatives such as diglucosly-beta-cyclodextrin; sulfoalkyl derivatives such as sulfobutylether-beta-cyclodextrin; and carboxymethylated derivatives such as carboxymethyl-beta-cylcodextrin. Other types of chemical modifications, known to those in the art, are also included within the scope of this invention.

The cyclodextrin complex often confers properties of improved solubility, dispersability, stability (chemical, physical and microbiological), bioavailability and decreased toxicity on the guest molecule (here, the derivative of the present invention).

There are a number of ways known in the art to produce a cyclodextrin complex. Complexes may be produced, for example, by using the following basic methods: stirring the constituents of the composition into an aqueous or mixed aqueous-organic solution of the cyclodextrin, with or without heating; kneading, slurrying or mixing the cyclodextrin and the present composition in a suitable device with the addition of an appropriate quantity of aqueous, organic or mixed aqueous-organic liquid, with or without heating; or by physical admixture the cylcodextrin and the composition of the present invention using a suitable mixing device. Isolation of the inclusion complex so formed may be achieved by co-precipitation, filtration and drying; extrusion/ spheronisation and drying; subdivision of the moist mass and drying; spray drying; lyophilization or by other suitable techniques

depending on the process used to form the cyclodextrin complex. A further optional step of mechanically grinding the isolated solid complex may be employed.

These cyclodextrin complexes further enhance the solubility and dissolution rate and increase the stability of the compositions. For a review of cyclodextrin complexation, please refer to 46.

Complexation with Bile Salts

Bile acids, their salts and conjugated derivatives, suitably formulated, may be used to solubilize the compositions of the present invention, thereby improving the solubility and dispersion characteristics of these compositions. Examples of suitable bile acids include: cholic acid, chenodeoxycholic acid, deoxycholic acid, dehydrocholic acid, and lithocholic acid. Examples of suitable bile salts include: sodium cholate, sodium deoxycholate and their other salt forms. Examples of suitable conjugated bile acids include: glycochenodeoxycholic acid, glycholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid and their salts.

A suitable system for further enhancing the solubility of the compositions of the present invention consists of the constituents of the composition plus one or more bile acids, salts or conjugated bile acids. Further materials may be added to produce formulations having additional solubilization capacity. These materials include, but are not limited to: phospholipids, glycolipids and monoglycerides. These ingredients may be formulated either in the solid phase or by the use of suitable solvents or carrier vehicles, with appropriate isolation and, optionally, particle size reduction using techniques described hereinabove.

Since bile acids and their derivatives have an unpleasant taste and may be irritating to the mucous membranes of the stomach and upper regions of the gastro-intestinal tract, a suitable enteric coating may be applied to the solid formulation particulates, using techniques known to those skilled in the art. Typical enteric coatings include, inter alia: cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate, polyvinylacetate phthalate,

acrylate polymers and th ir derivatives (e.g. appropriate members of the Eudragit™ series), ethylcellulose or combinations thereof. Additional excipients may be added to the coating formulation to modify membrane functionality or to aid in the coating process (e.g. surfactants, plasticisers, channeling agents, permeability modifiers and the like). Coating formulation vehicles may comprise aqueous or organic systems, or mixtures of both.

Hydrotropic Complexation

Compounds which are capable of opening up the water structure associated with hydrophobic (lipophilic) and other molecules are referred to as hydrotropes. These compounds may be used to enhance further the aqueous solubility of the compositions. Examples of hydrotopes include, inter alia, sodium benzoate, sodium hydroxybenzoates, sodium salicylate, nicotinamide, sodium nicotinate, sodium gentisate, gentisic acid ethanolamide, sodium toluates, sodium aminobenzoates, sodium anthranilate, sodium butylmonoglycolsulfate, resorcinol and the like.

Complex formation, which is non-covalent in nature, may be achieved by mixing the composition and the hydrotope or mixtures thereof in a suitable liquid vehicle, which may be aqueous, organic or a combination of both. Additional excipients such as surfactants, polyols, disaccharides etc.. may be added to facilitate complexation or to aid in dispersability. The resultant complex is isolated as a dry powder by any process known in the art (co-precipitation and drying, evaporation of the liquid vehicle, spray drying, lyophilization etc..). Particle size may be reduced by any standard technique such as those described previously herein, if desired. The resultant hydrotope complex may be used without further modification or may be compounded into a variety of other formulations or vehicles as required.

Methods of Use

The composition of the present invention may be administered to animals, in particular humans, directly and without further modification or alternatively may be incorporated into various vehicles as described further below in order to treat an/or prevent CVD, its underlying conditions as well as oth r disorders such as diabetes type II, hypert nsion and visceral obesity. In populations which are considered "high-risk" for CVD, it is

contemplated that the composition of the present invention b used in primary, secondary and tertiary treatment programs.

Without limiting the generality of the foregoing, the composition of the present invention may be admixed with various carriers or adjuvants to assist in direct administration or to assist in the incorporation of the composition into foods, beverages, nutraceuticals or pharmaceuticals. In order to appreciate the various possible vehicles of the delivery of the composition, the following examples are provided.

1) Pharmaceutical Dosage Forms:

It is contemplated within the scope of the present invention that the composition of the present invention may be incorporated into various conventional pharmaceutical preparations and dosage forms such as tablets (plain and coated) for use orally, bucally or lingually, capsules (hard and soft, gelatin, with or without additional coatings) powders, granules (including effervescent granules), pellets, microparticulates, solutions (such as micellar, syrups, elixirs and drops), lozenges, pastilles, ampuls, emulsions, microemulsions, ointments, creams, suppositories, gels, transdermal patches and modified release dosage forms together with customary excipients and/or diluents and stabilizers.

The composition of the present invention, adapted into the appropriate dosage form as described above may be administered to animals, including humans, orally, by injection (intra-venously, subcutaneously, intra-peritoneally, intra-dermally or intra-muscularly), topically or in other ways. Although the precise mechanism of action is unclear, the composition of the present invention, administered intra-venously, lowers serum cholesterol. It is believed that certain phytosterol-based compositions may have, in addition to the role as an inhibitors of cholesterol absorption in the intestine, a systemic effect on cholesterol homeostasis through bile acid synthesis, enterocycte and biliary cholesterol excretion, bile acid excretion and changes in enzyme kinetics and cholesterol transport between various compartments within the body (PCT/CA97/00474 which was published on January 15, 1998). See also paper to Peter Jones (under publication).

2) Foods/Beverages/Nutraceuticals:

In another form of the present invention, the composition of the present invention may be incorporated into foods, beverages and nutraceuticals, including, without limitation, the following:

1) Dairy Products –such as cheeses, butter, milk and other dairy beverages, spreads and dairy mixes, ice cream and yoghurt;

2) Fat-Based Products–such as margarines, spreads, mayonnaise, shortenings, cooking and frying oils and dressings;

3) Cereal-Based Products–comprising grains (for example, bread and pastas) whether these goods are cooked, baked or otherwise processed;

4) Confectionaries–such as chocolate. candies, chewing gum, desserts, non-dairy toppings (for example Cool Whip™), sorbets, icings and other fillings;

5) Beverages– whether alcoholic or non-alcoholic and including colas and other soft drinks, juices, dietary supplement and meal replacement drinks such as those sold under the trade-marks Boost™ and Ensure™; and

6) Miscellaneous Products–including eggs, processed foods such as soups, pre-prepared pasta sauces, pre-formed meals and the like.

The composition of the present invention may be incorporated directly and without further modification into the food, nutraceutical or beverage by techniques such as mixing, infusion, injection, blending, immersion, spraying and kneading. Alternatively, the composition may be applied directly onto a food or into a beverage by the consumer prior to ingestion. These are simple and economical modes of delivery.

EXAMPLES

The present invention will be described by way of the following non-limiting examples.

Example 1--Therapeutic Assessment of Composition

Materials and Methods

Animals and Diets:

Twenty eight male apo E-deficient (apo E-KO) mice were purchased from the Jackson Laboratory, Bar Harbor, MN. After a 10-day adaptation period, the mice were divided into 4 experimental groups matched with body weight and plasma cholesterol and triglyceride concentrations. Group 1 (n=8) was given a western-type diet (WD) containing 9% (w/w) fat and 0.2% (w/w) cholesterol (Sigma) and served as the control group. Group 2 (n=8) was given the above mentioned WD supplemented with 1% (w/w) n-3 fatty acid (Lysaker, Norway); groups 3 and 4 (n=6 each) were given the same diet as group 2, but supplemented with either 1% (w/w) FCP-3P1 (group 3) or FCP-3P2 (group 4), respectively. Plant sterols (FCP-3P1 and FCP-3P2) are compositions within the scope of the present invention and were produced by Forbes Medi-Tech Inc. Vancouver, BC. The animals were fed the above-mentioned diets for 8 weeks. Plasma lipid profiles were determined at baseline and at 4-week intervals, while body weight was measured weekly. Preparation of all experimental diet was based on previously established methods (47,48) and performed in the JR laboratories, Burnaby, BC. The experimental diets were tested for auto-oxidation by measurements of total free fatty acids and peroxide value just after preparation and 2 weeks later. Hence, the diets were prepared every other week. The experimental protocol was approved by the Animal Care Committee at the University of British Columbia, Vancouver, BC.

Measurements of total free fatty acid and peroxide values in the experimental diets:

Total free fatty acids in the diet was determined by a titration method. Briefly, the dietary fat was extracted followed by addition of phenolphthalein and titration with 0.1N NaOH. Similarly, the rancidity (peroxide value) of the diets was estimated by a titration method using Na2S2O3. These analyses were carried out in a certified laboratory (JR Laboratories Inc., Burnaby, BC).

M asurements of plasma cholesterol and triglycerides levels:
The animals were bled from the tail at the outset and during the experimental course as described previously (47, 48). Plasma was separated by centrifugation and used for the measurement of total cholesterol and triglycerides concentrations using an enzymatic method in the clinical laboratory at the St. Paul's Hospital, Vancouver, BC. Plasma lipid measurements were performed in a blind fashion.

Statistical analysis:
One way ANOVA followed by the application of Tukey test was performed to detect statistically significant differences between the results of all 4 groups of the mice by using SPSS software.

Results
Total free fatty acids and proxide values of the experimental diets:
Auto-oxidation of the experimental diets were evaluated just after preparation and 2 weeks later by measuring total free fatty acids and peroxide value. The results are summarized in Table 1. As is evident, the 2-week storage of prepared diets was not associated with auto-oxidation. Therefore, we decided to prepare the experimental diets every other week.

Body weight:
The animals' body weights were measured weekly at the baseline and during the experimental course. Table 2 demonstrates the weekly mean body weights of all 4 experimental groups of the mice. The data indicate that all groups of mice have had a comparable body weight gain during the experimental course.

Plasma lipid profiles:
Figure 1 shows total cholesterol levels in plasma of 4 groups of the animals at baseline and during the experimental course (weeks 4 and 8). All mice had comparable total cholesterol concentrations at baseline. The Western-type diet markedly increased plasma total cholesterol concentrations (control group). Addition of 1% (w/w) n-3 fatty acid did not decrease plasma total cholesterol lev ls as compared to controls. One other hand, combination of n-3 fatty acid and plant sterols

significantly decreased plasma total cholest rol levels as compared to controls by the week 8 of the experiment. This significant decrease in plasma total cholesterol levels was associated with a small but statistically significant increase in plasma triglycerides levels at the week 8 of the experiment (Figure 2).

Comments

These preliminary data indicate that the addition of combination of n-3 fatty acid and plant sterols to a Western-type diet is tolerated very well in apo E-deficient mice. This combination therapy is associated with a marked decrease in total cholesterol levels as compared to controls by the week 8 of the experiment. We have previously shown that reduction in plasma total cholesterol levels by plant sterols in apo E-KO was associated with a significant decrease in atherosclerosis (47, 48). Similarly, administration of high dose of n-3 fatty acid to mice was also associated with a significant decrease in atherosclerosis (49). The present study indicates that combination therapy with lower doses of both plant sterols and n-3 fatty acids significantly reduces plasma total cholesterol levels during 8 week of treatment. It is estimated that this reduction in plasma total cholesterol levels would be associated with a significant decrease in atherosclerosis development in apo E-KO mice.

Group#	Bas line	W1	%increase	W2	%incr ase	W3	%increase
1	19.1	23.4	22.5	24.6	28.8	26.6	39.3
	19	23.3	22.6	25.3	33.2	26.6	40.0
Controls	19.5	22.8	16.9	23.4	20.0	25.1	28.7
	19.6	22.3	13.8	23.8	21.4	25.7	31.1
	20.1	22.5	11.9	23.7	17.9	25.5	26.9
	21.8	24	10.1	25.4	16.5	27.2	24.8
	19.6	24.3	24.0	25.3	29.1	26.8	36.7
	20.4	23	12.7	25.8	26.5	27.2	33.3
Mean	**19.9**	**23.2**	**16.8**	**24.7**	**24.2**	**26.3**	**32.6**
Sd	**0.9**	**0.7**	**5.5**	**0.9**	**6.0**	**0.8**	**5.7**
2	17.6	21.5	22.2	23.9	35.8	25	42.0
	20.7	23.7	14.5	25.9	25.1	27.8	34.3
1% n-3	19.6	23.5	19.9	24.7	26.0	25.6	30.6
Fatty acids	18.5	22.3	20.5	23.8	28.6	25.2	36.2
	21.1	24.4	15.6	26.1	23.7	27.1	28.4
	19.5	22.9	17.4	24.6	26.2	26.2	34.4
	19.6	24.5	25.0	25.6	30.6	27.4	39.8
	21	24.8	18.1	25.9	23.3	28	33.3
Mean	**19.7**	**23.5**	**19.2**	**25.1**	**27.4**	**26.5**	**34.9**
Sd	**1.2**	**1.2**	**3.5**	**0.9**	**4.2**	**1.2**	**4.6**
3	20.8	23.3	12.0	24.8	19.2	27	29.8
	15.4	19.3	25.3	21.7	40.9	23.6	53.2
1% n-3	18.2	22	20.9	23.9	31.3	26.8	47.3
Fatty acids	19.9	23.2	16.6	24.6	23.6	26.6	33.7
+	20.7	23	11.1	24.8	19.8	26.7	29.0
1% FCP-3P1	22.9	24.9	8.7	26.5	15.7	28.2	23.1
Mean	**19.7**	**22.6**	**15.8**	**24.4**	**25.1**	**26.5**	**36.0**
Sd	**2.6**	**1.9**	**6.4**	**1.6**	**9.4**	**1.5**	**11.7**
4	20.6	23.2	12.6	23.8	15.5	26.1	26.7
1% n-3	19.2	23.5	22.4	24.7	28.6	26.8	39.6
fatty acids	20	24.2	21.0	25.4	27.0	27.7	38.5
+	20.5	23.2	13.2	25.3	23.4	26.8	30.7
1% FCP-3P2	19.6	23	17.3	24.6	25.5	26.3	34.2
	19.3	22.9	18.7	24.9	29.0	26.2	35.8
Mean	**19.9**	**23.3**	**17.5**	**24.8**	**24.9**	**26.7**	**34.2**
Sd	**0.6**	**0.5**	**4.0**	**0.6**	**5.0**	**0.6**	**4.9**

Group#	W4	%incr ase	W5	%increase	W6	%increase
1	27.8	45.5	27.6	44.5	28.1	47.1
	27.4	44.2	27.5	44.7	28.3	48.9
Controls	26.5	35.9	26.9	37.9	27.5	41.0
	25.5	30.1	26.2	33.7	26.5	35.2
	25.5	26.9	26.1	29.9	26.2	30.3
	27.1	24.3	27.3	25.2	27.6	26.6
	28	42.9	28.7	46.4	29.5	50.5
	28.2	38.2	28.9	41.7	29.4	44.1
Mean	27.0	36.0	27.4	38.0	27.9	40.5
Sd	1.1	8.2	1.0	7.7	1.2	8.9

2	26.1	48.3	26.8	52.3	27.5	56.3
	28.8	39.1	29.3	41.5	29.8	44.0
1% n-3	26.7	36.2	27.2	38.8	27.7	41.3
Fatty acids	25.8	39.5	25.8	39.5	25.7	38.9
	28.2	33.6	28.8	36.5	30.4	44.1
	27.1	39.0	27.5	41.0	29.1	49.2
	27.9	42.3	28.5	45.4	29.3	49.5
	27.9	32.9	28.6	36.2	29.6	41.0
Mean	27.3	38.9	27.8	41.4	28.6	45.5
Sd	1.1	5.0	1.2	5.3	1.6	5.7

3	27.8	33.7	28.9	38.9	30.3	45.7
	25	62.3	25.9	68.2	26.4	71.4
1% n-3	27	48.4	27.3	50.0	28.1	54.4
Fatty acids	26.8	34.7	27.6	38.7	28.2	41.7
+	27.2	31.4	28.3	36.7	28.5	37.7
1% FCP-3P1	29.1	27.1	29.8	30.1	30.2	31.9
Mean	27.2	39.6	28.0	43.8	28.6	47.1
Sd	1.3	13.2	1.4	13.6	1.5	14.1

4	27.6	34.0	28.3	37.4	29.5	43.2
1% n-3	26.9	40.1	27.2	41.7	27.8	44.8
fatty acids	28.3	41.5	29.3	46.5	29.7	48.5
+	28	36.6	29	41.5	29.7	44.9
1% FCP-3P2	27	37.8	27.6	40.8	28.5	45.4
	27	39.9	27.6	43.0	28.8	49.2
Mean	27.5	38.3	28.2	41.8	29.0	46.0
Sd	0.6	2.8	0.8	3.0	0.8	2.3

Gr up#	W7	%increase	W8	%increase	W9	%increase
1	28.3	48.2	28.8	50.8	28.5	49.2
	28.5	50.0	28.9	52.1	29.2	53.7
Controls	27.1	39.0	28.7	47.2	29.1	49.2
	26.6	35.7	27.6	40.8	27.6	40.8
	26.6	32.3	27.1	34.8	27.9	38.8
	27.9	28.0	28.4	30.3	28.5	30.7
	29.4	50.0	30.6	56.1	30.9	57.7
	30.2	48.0	30.4	49.0	30.3	48.5
Mean	**28.1**	**41.4**	**28.8**	**45.1**	**29.0**	**46.1**
Sd	**1.3**	**8.8**	**1.2**	**9.0**	**1.1**	**8.7**

2	27.7	57.4	29	64.8	29.4	67.0
	30.2	45.9	31	49.8	31.4	51.7
1% n-3	27.6	40.8	28.1	43.4	27.9	42.3
Fatty acids	24.9	34.6	24.5	32.4	24.4	31.9
	31	46.9	31.2	47.9	31.3	48.3
	29.5	51.3	29.5	51.3	29	48.7
	29.4	50.0	30.6	56.1	30.8	57.1
	29.8	41.9	30.5	45.2	30.6	45.7
Mean	**28.8**	**46.1**	**29.3**	**48.9**	**29.4**	**49.1**
Sd	**1.9**	**7.1**	**2.2**	**9.5**	**2.3**	**10.3**

3	30.5	46.6	30.5	46.6	30.6	47.1
	26.6	72.7	27.2	76.6	27.6	79.2
1% n-3	28.7	57.7	28.8	58.2	29.2	60.4
Fatty acids	28.8	44.7	28.9	45.2	28.7	44.2
+	28.6	38.2	29.5	42.5	29.8	44.0
1% FCP-3P1	30.2	31.9	31.2	36.2	31.2	36.2
Mean	28.9	48.6	29.4	50.9	29.5	51.9
Sd	1.4	14.6	1.4	14.5	1.3	15.6

4	31.3	51.9	31.5	52.9	31.6	53.4
1% n-3	27.9	45.3	28.5	48.4	28.6	49.0
fatty acids	30.2	51.0	31.4	57.0	31.6	58.0
+	29.3	42.9	31.2	52.2	31.1	51.7
1% FCP-3P2	29.2	49.0	29.4	50.0	29.1	48.5
	28.1	45.6	28.4	47.2	28.5	47.7
Mean	29.3	47.6	30.1	51.3	30.1	51.4
Sd	1.3	3.6	1.5	3.5	1.5	3.9

	Baseline		4 weeks		8 weeks	
Group#	TC	TG	TC	TG	TC	TG
1	16.5	0.8	30.8	0.6	36.86	1.2
	10.0	1.3	34.1	0.5	30.45	0.62
	9.6	0.4	28.2	1.1	33.3	0.9
	13.8	0.8	35.3	1.1	44.4	1.64
	9.9	0.9	37.8	0.8	39	1.36
	10.5	0.9	37.6	0.8	37.1	0.85
	13.1	0.8	23.2	0.6	24.91	0.49
	14.9	2.1	29.6	0.9	29.31	0.78
Mean	**12.3**	**1.0**	**32.1**	**0.8**	**34.4**	**1.0**
Sd	**2.6**	**0.5**	**5.0**	**0.2**	**6.2**	**0.4**
2	14.7	0.9	46.8	1.5	39	2.17
	12.3	0.9	35.5	4.1	31.65	2.5
	12.8	0.9	37.4	3.3	42.3	3.28
	13.9	0.8	36.3	2.5	25.93	1.28
	12.0	1.0	31.0	2.6	42.55	2.74
	13.6	0.8	26.6	2.1	29.31	2.18
	11.7	0.8	27.8	0.9	24.11	1.54
	10.6	1.2	31.5	2.2	24.83	1.24
Mean	**12.7**	**0.9**	**34.1**	**2.4**	**32.6**	**2.1**
Sd	**1.3**	**0.1**	**6.4**	**1.0**	**7.8**	**0.7**
9	11.3	0.5	17.5	2.9	19.81	3.61
	13.6	0.3	16.4	2.4	17.15	4.75
	13.9	0.7	15.8	4.5	19.05	6.38
	10.6	0.6	12.4	2.6	20.31	5.13
	9.4	1.4	18.0	3.5	17.96	4.56
	12.6	2.3	20.3	4.6	18.32	4
Mean	**11.9**	**1.0**	**16.7**	**3.4**	**18.8**	**4.7**
Sd	**1.8**	**0.8**	**2.6**	**1.0**	**1.2**	**1.0**
11	14.9	0.9	32.5	9.7	35.84	8.92
	9.4	0.7	22.4	4.9	23.04	5.2
	9.9	1.4	16.7	3.7	16.4	2.92
	12.2	1.9	18.3	4.0	17.68	2.72
	11.5	1.5	17.8	3.4	20.2	4.08
	9.5	0.7	Missing	1.4	16.68	2.6
Mean	**11.2**	**1.2**	**21.5**	**4.5**	**21.6**	**4.4**
Sd	**2.1**	**0.5**	**6.5**	**2.8**	**7.4**	**2.4**

	control	n-3 FA	1% 3P1+n-3	1%3P2+n 3
week 0	12.3	12.7	11.9	11.2
week 4	32.1	34.1	16.7	21.5
week 8	34.4	32.5	18.8	21.6

	control	n-3 FA	1% 3P1+n-3	1%3P2+n 3
week 0	1	0.9	1	1.2
week 4	0.8	2.4	3.4	4.5
w ek 8	1	2.1	4.7	4.4

Table 1: Total free fatty acid and peroxide value in experimental diets just after (week preparation and 2-weeks later (week 2).

Diet	Total free fatty acids (% in diet sample)		Peroxide value (meq/kg diet sample	
	Week 0	Week 2	Week 0	Week 2
Control	0.45	0.35	0.79	0.2
1% n-3 fatty acids	0.44	0.41	0.92	0.7
1% n-3 fatty acids +1% FCP-3P1	0.33	0.40	0.79	0.8
1% n-3 fatty acids + 1% FCP-3P2	0.34	0.44	1.17	0.9

Table 2: Mouse body weight (g) at outset and during the experimental course

Groups (n)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (8)	19.9	23.2	24.7	26.3	27	27.4	27.9	28.1	28.8
1% n-3 fatty acid (8)	19.7	23.5	25.1	26.5	27.3	27.8	28.6	28.8	29.3
1% n-3 fatty acid + 1% FCP-3P1 (6)	19.7	22.6	24.4	26.5	27.2	28	28.6	28.9	29.4
1% n-3 fatty acid + 1% FCP-3P2 (6)	19.9	23.3	24.8	26.7	27.5	28.2	29	29.3	30.1

Discussion

The purpose of the above study was to examine the relationship between dietary intake of tall oil (16% sitostanol) and vegetable oil derived plant sterols (77.8% sitostanol) and omega-3 fatty acids on plasma cholesterol and triglyceride levels. Disturbances in the plasma concentration of triacylglycerol –rich lipoproteins in the postabsorptive and postprandial periods are associated with increase atherogenic risk (50). In Western population, most deaths from coronary heart disease occur

among individuals whose cholesterol levels ar averag or below average, therefor , the benefit in lowering of cholesterol levels in this patient group has been questioned (51). The recent study provided the first evidence that postprandial lipoprotein rapidly penetrates arterial tissue with significant quantities find in 5 to 20 min. in carotid media region (52). Thus, while therapeutic intervention is focusing on the reduction of LDL - cholesterol, there are other lipid factors that can affect the progression of the disease.

Since VLDL and chylomicron share a common saturable lipolytic system, delayed conversion of VLDL to LDL would be reflected in the accumulation of intermediate density lipoproteins and chylomicron remnants. The accumulation of VLDL and chylomicron remnants and its lipolytic products is influenced by variation in apo -E and lipoprotein lipase and various apolipoproteins. Transgenic mouse studies have been
increasingly utilized to answer specific question, which relates to human disease. Data from apo E study suggest that observed regression of atherosclerosis associated with that tall oil derived plant sterols feeding increase the utilization of lipoprotein lipase, inhibit hepatic lipase and decrease each of LDL, hepatic and plaque cholesterol levels (53, 54).

Twenty years ago, Bang et al. suggested that it was the high omega - 3 fatty acid content in the Eskimos diet that accounted for the low rate of ischemic heart disease (55). The hypolipidemic, antithrombotic and antiatheromatous aspects of omega -3 fatty acids have been extensively studied. The hypolipidemic effects of omega - 3 fatty acids consist of lowering serum postprandial triglyceride concentration (56), reduce the rate of hepatic secretion of VLDL and increase the VLDL triglyceride fractional catabolic rate (57, 58), reverse the carbohydrate-induced hypertriglyceridemia (59) and while serum saturated fatty acids are directly, omega - 3 fatty acids are inversely correlated with coronary artery disease (60). Omega -3 fatty acids biological effects mechanism involve rapid transcription alteration of specific genes and cell signaling transduction pathways (60, 61).

Example 1 examined relation between dietary intake of tall oil and v getable oil derived plant sterols and omega - 3 fatty acids on plasma cholesterol and triglycerides level. The dietary plant sterol enrichment in human clinical studies consistently decreases cholesterol plasma levels but has no effect on plasma triglyceride (62). The apo E deficient mice accumulates plasma chylomicron remnants requiring apo -E for receptor uptake.

This example clearly demonstartes a significant coeffect of the combined administration of phytosterols and/or phytostanols and omega-3 PUFAs on both plasma cholesterol and triglycerides levels. The following is an analysis of the experimental results.

In this animal model there is statistically significant dietary plant sterol, omega-3 fatty acids dependent increase in plasma fasting triglyceride. As the tissue omega -3 fatty acids content increase, the plasma triglycerides levels stabilize, or start to show regression. Plant sterols decrease plasma cholesterol levels in a dose dependent manner, with the tall oil derived composition being more efficient than vegetable-derived sources. The omega - 3 fatty acids had an inconsistent non-significant inhibitory effect on plant sterol plasma cholesterol lowering, with the exception of the 0.5 % plant sterols - omega-3 fatty acids dietary supplement, where omega- 3 fatty acids suppress plant sterol cholesterol lowering effect with increase in fasting triglycerides. The plant sterol is triglyceride and omega-3 fatty acids cholesterol neutral. The study did not demonstrate any significant weight changes between the experimental groups.

There is an indication that the administration of a combination of plant sterols - omega -3 fatty acids has three phase effects: rapid accumulation of plasma triglycerides with gradual slope regression; decrease in initial cholesterol regression followed by gradual slope regression and positive correlation between cholesterol and triglyceride plasma levels. In the absence of apo E, these results are suggestive of inhibition of VLDL and chylomicron remnants catabolism due to rapid saturation of lipoprotein lipase catabolic pathway. Furthermore, omega-3 fatty acids in clinical studies decrease both plasma TG and VLDL. These arguments suggest, that omega-

3 fatty acids - plant sterol study results are based on the accumulation of postprandial lipoprot ins (VLDL, chylomicron remnants) due to enhanced triglyceride rich lipoprotein particles turnover with impaired CETP ,apoE-mediated plasma triglyceride rich lipoproteins accumulation . This conclusion is also supported by no weight gain observed in plant sterol - omega -3-fatty acids study. This finding is important, since an increase in postprandial lipoprotein turnover could decrease free radical formation and endothelial dysfunction.The plasma triglyceride slope regression could be mediated by changes in plasma apoproteins C -II ,C-III ratio,and possibly by PPAR intracellular receptor mediated mechanism transducing the signal to genome. The PPAR alpha is better activated by PUFA (63).

Example 2- Formulations

In order to evaluate the applicability of various formulation approaches for one phytosterol/phytostanol combination (FCP-3P1) and omega-3 fatty acids, simplified examples of potential formulae were investigated. Unless otherwise stated, FCP-3P1 Batch FM-pH-42 (composition: campesterol 14.35%, campestanol 3.07%, β-sitosterol 54.67%, and sitostanol 15.76%) was used in the formulation work. Content uniformity data was referenced to the total phytosterol content of the batch, ie 87.85%.

The flaxseed oil used in this study was provided by L.V. Lomas Ltd., Delta, B.C., Sample # 36077. Literature accompanying the product stated that it contained, among other components, natural vitamin E 2.5 mg/g. The reported Linolenic acid (omega-3) content was 57%, Linoleic acid (Omega-6) was 16%, and Oleic acid (Omega-9) was 18%. Total Omega-3 plus Omega-6 fatty acid content was 73%. The hempseed oil was provided by Hempola, Mississauga, Ont. Literature provided by the manufacturer indicated that the Linolenic acid (Omega-3) content was 16.61%, Linoleic acid (Omega-6) content was 55.75%, and the Oleic acid (Omega-9) content was 12.84%. Total Omega-3 plus Omega-6 fatty acid content was 72.36%. Thus the flaxseed oil contains a significantly higher proportion of Omega-3 fatty acid than the hempseed oil.

Solubility of FCP-3P1 in flax and hempseed oils

This was determined by adding 100mg of the FCP-3P1 to 2mL of each of the fatty acid oils in a 10mL glass screw cap tube. Samples were equilibrated by vortexing (VWR Multi-tube Vortexer, setting 2) at either 21° C or 60° C (duplicate samples at each temperature and for each oil) for 24 hours. The tubes were allowed to attain room temperature (21° C) then centrifuged at 3000x for 5 minutes and independently sampled for analysis by gas chromatography (GC-FID), using a cholestane internal standard. Results are presented in Table 3.

Table 3: Solubility of FCP-3P1 in flax and hempola oils

Oil	FCP-3P1 (FM-PH-42) solubility (mg/mL)[a]	
	21° C	60° C*
Flax	13.79	12.07
Hempola	8.54	9.70

[a] Given as the sum of major phytosterol components (campestanol + campesterol + β-sitosterol + sitostanol = 87.85% of sample weight)

* Samples were allowed to cool to 21° C prior to analysis

It will be noted that FCP-3P1 solubility was somewhat higher in flaxseed oil than hempseed oil, at both test temperatures.

Macroemulsion formulation

A 10% w/w solution of FCP-3P1 was prepared by adding 1.5005 g of FCP-3P1 to 2.0708 g flaxseed oil and 11.4451 g soybean oil and heating to 63° C to give a clear solution. Into 10 mL of this solution was dissolved 0.7464 g of Span 60 [polyoxyethylene-(20)-sorbitan monostearate]. This constituted the oil phase. This surfactant has a Hydrophile-Lipophile Balance (HLB) value of 4.7 ± 1.0.

The aqueous phase consisted of a 15 mL solution of 0.7578 g Tween 40 [polyoxyethylene-(20)-sorbitan monopalmitate] and 0.7508 g EDTA (ethylene diamine tetra-acetic acid) in distilled de-ionized water. Tween 40 has an HLB value of 15.6 ± 1.0. Both oil and aqueous phases were individually heated to 70° C, combined and

vigorously mixed using a Polytron Model PCV II mixer, on the high sp ed setting, for 1 minut . The product was left to cool to ambient temperature. This gave an oil in water emulsion, with an oil (dispersed) phase of 40% in an aqueous continuous phase, containing a dual surfactant system having an overall HLB of 10.0 ± 1.0 and an active loading of ca 4% w/v. Drug was in the oil phase.

Phase Separation Assessment

15 mL of emulsion was poured into a graduated centrifuge tube, which was subsequently sealed. Daily visual inspection over 4 days indicated no phase separation.

pH

The measured pH of the system was 4.68.

Oil Phase Droplet Size

This parameter was evaluated using an optical microscope equipped with a calibrated eyepiece under polarizing conditions. Sample preparation involved diluting 1 part of emulsion with 4 parts water and examining on a microscope slide, under a cover slip, at 400x magnification. The dispersed oil phase consisted of droplets ranging from ca 2.5 —15 microns and no evidence of FCP-3P1 crystallization was observed.

FCP-3P1 Content Uniformity Determination

This was assessed on 6 samples, removed from the bulk according to a pre-determined sample plan. Each sample (0.5 mL) was extracted by vortexing for 10 minutes with dichloromethane (DCM, 5 mL), followed by centrifugation at 4000 rpm for 2 min to separate the two phases. The analytical sample was withdrawn from the DCM layer and assayed by GC-FID, using a cholestane internal standard. Results are reported in Table 4.

Table 4: Content uniformity of FCP-3P1 in macroemulsion formulation

Sample #	FCP-3P1 in emulsion (mg/mL)*
1	18.95
2	19.70
3	18.68
4	19.39
5	18.52
6	18.41
Mean	18.94 (53.9% of theoretical)
Standard Deviation	0.51
Theoretical content (of test sample)	35.15

* Reflects total of major phytosterols only (campestanol + campesterol + β-sitosterol + sitostanol = 87.85% of sample weight)

Content uniformity is acceptable (18.94 ± 0.51 mg/mL) and indicates satisfactory emulsion homogeneity. Recovery is low (54%) and probably reflects a combination of incomplete extraction of active from emulsion by DCM and pipetting errors.

This dosage delivery system has successfully incorporated both FCP-3P1 and flaxseed oil (Omega fatty acid source) in a single formulation.

Example 3- Solutions and Dispersions (oil-based)

An oil-based soft gelatin capsule formulation was obtained by taking the oil phase from the macroemulsion, with or without modification, and filling the solution into a soft gelatin capsule. Potential modifications could include increasing the content of FCP-3P1, by forming a dispersion or paste; altering the ratio of FCP-3P1 (the phytosterol/stanol component) to omega fatty acids, with appropriate adjustment of the soybean oil diluent; inclusion of Tween 40 or other suitable surfactant at an appropriate level. In the event that a dispersion or paste is required, the particle size of the FCP-3P1 may be modified by milling in the dry state, or dispersed in some or all of the oil components (microfluidization), to achieve the desired end result.

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W Claim:

1. A composition for use in preventing and treating cardiovascular disease and other disorders which comprises one or more phytosterols, phytostanols or mixtures of both, and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

2. The composition of claim 1 wherein the phytosterols are esterified.

3. The composition of claim 1 wherein the phytosterols are selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized, isomeric forms and derivatives thereof.

4. The composition of claim 1 wherein the phytostanols are selected from the group consisting of sitostanol, campestanol, stigmastanol, brassicastanol, desmostanol, chalinostanol, poriferastanol, clionastanol and all natural or synthesized, isomeric forms and derivatives thereof.

5. The composition of claim 1 wherein the omega-3 polyunsaturated fatty acid is selected from the group consisiting of linolenic acid, eicosapentaenoic acid and docosahexenoic acid and all derivatives thereof.

6. The composition of claim 1 wherein the omega-3 polyunsaturated fatty acid is provided in the form of one or more marine oils.

7. The composition of claim 1 wherein the omega-3 polyunsaturated fatty acid is provided in the form of one or more plant oils.

8. The composition of claim 1 additionally comprising one or more omega-6 polyunsaturated fatty acids.

9. The composition of claim 1 additionally comprising one or more omega-6 polyunsaturated fatty acids provided in the form of plant oils.

10. The composition of claims 1 incorporated into a vehicle selected from the group consisting of a food, a beverage, a pharmaceutical and a nutraceutical.

11. A method of treating and preventing cardiovascular disease and other disorders in an animal which comprises administering to the animal a composition comprising one or more phytosterols, phytostanols or mixtures of both, and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

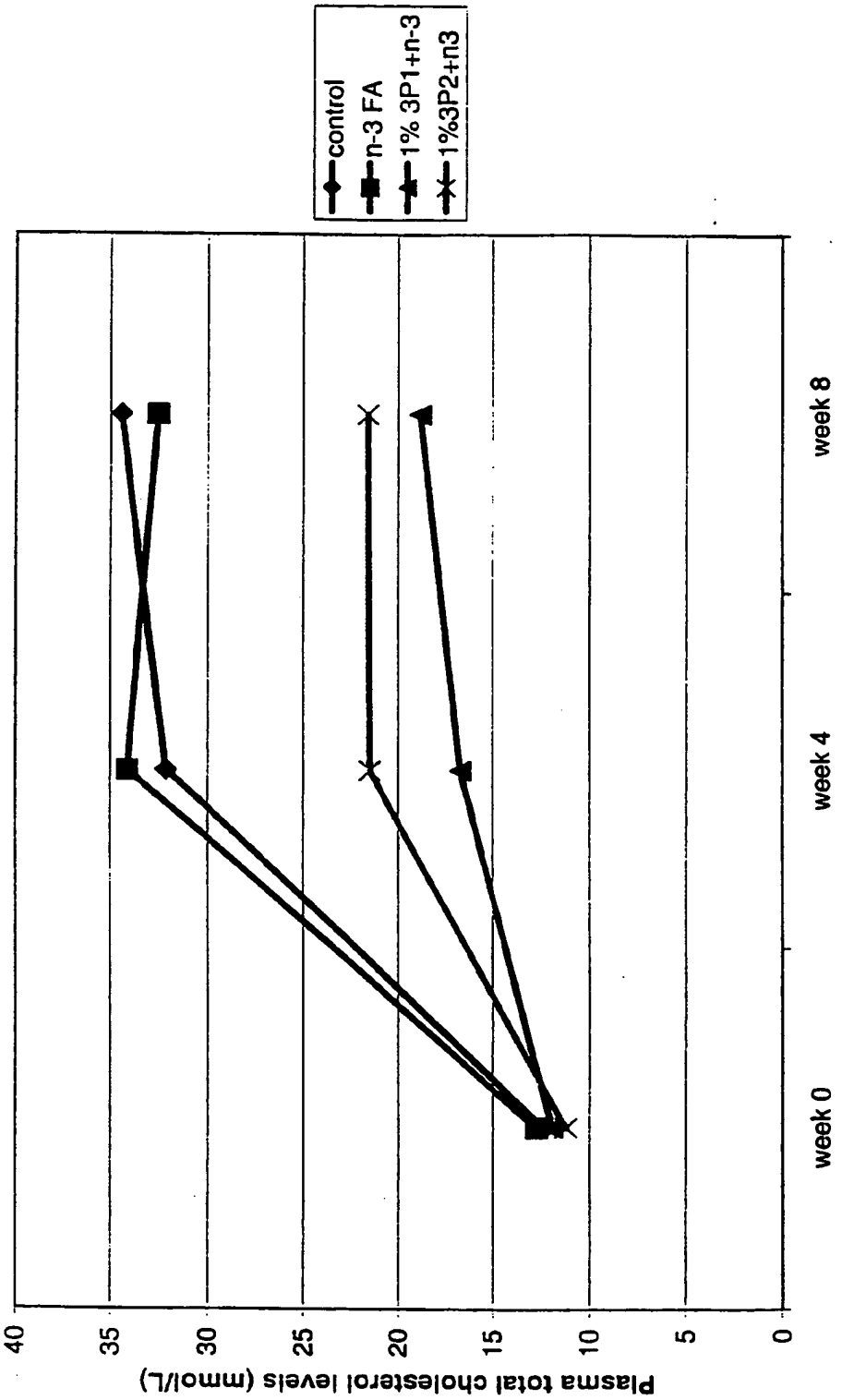

Figure 1

SUBSTITUTE SHEET (RULE 26)

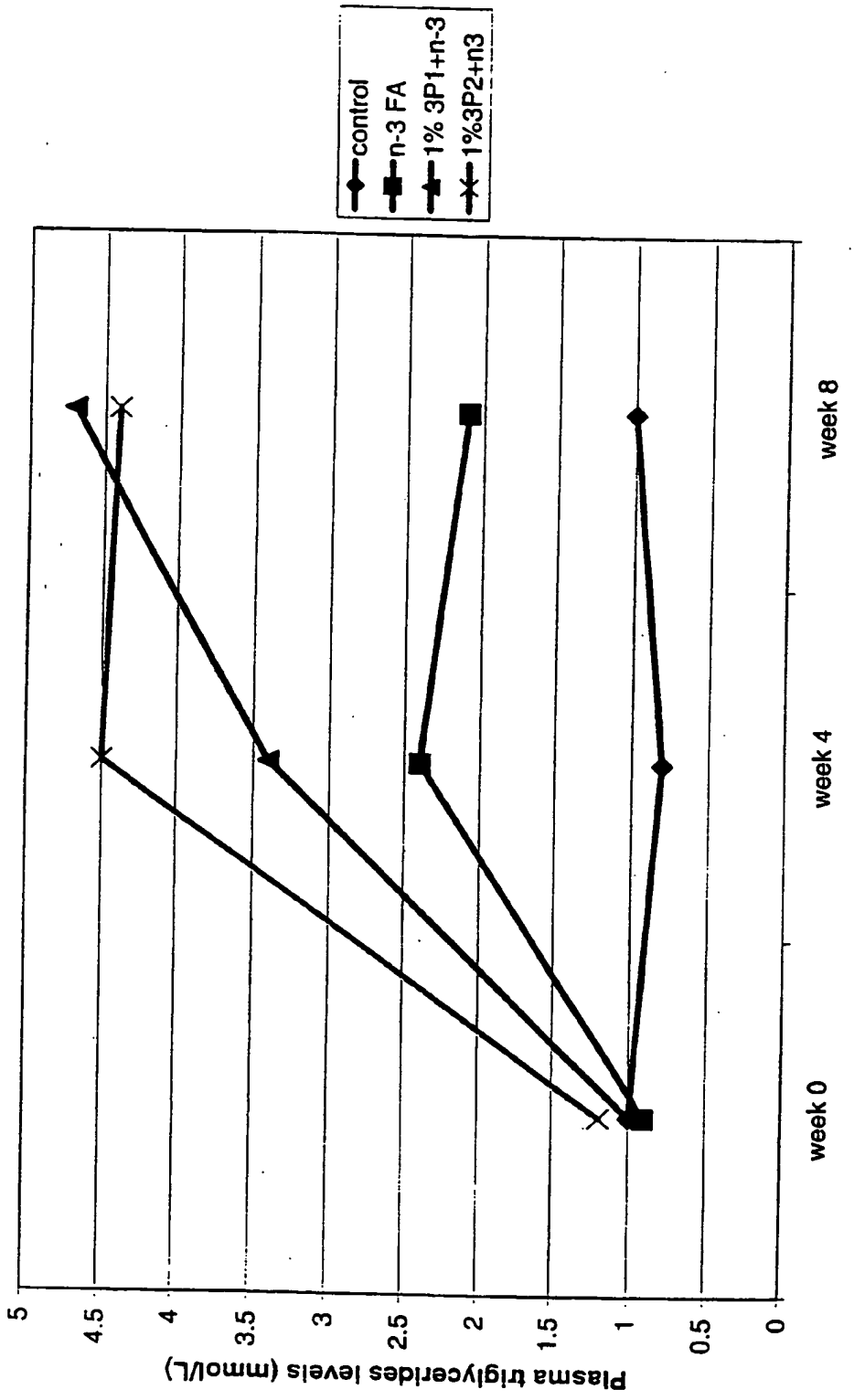
control
n-3 FA
1% 3P1+n-3
1%3P2+n3
Plasma triglycerides levels (mmol/L)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
week 0
week 4
week 8

Figure 2